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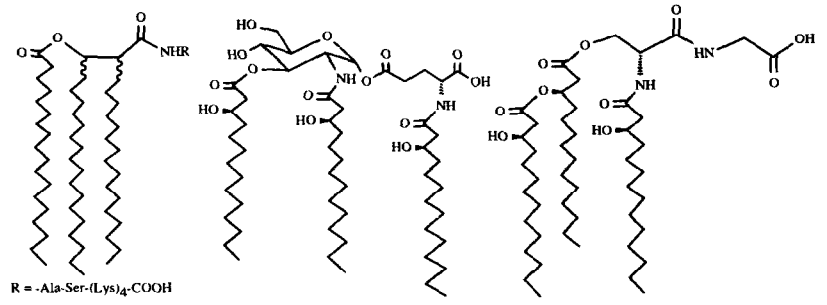
Synthesis of the 4 possible stereoisomers of 3-O-stearoyl C_{36}-corynomycolic acid and derived lipopeptides.

Jacques Eustache* and Alfred Grob

Sandoz Forschungsinstitut Wien, Brunner Strasse 59, A-1235 Wien (Austria)

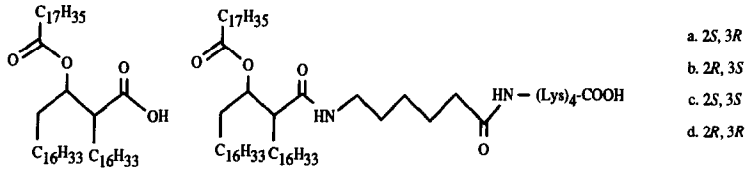
Abstract-A short synthesis of 3-O-stearoyl (*S,R*), (*R,S*), (*S,S*) and (*R,R*) C_{36}-corynomycolic acids is described. Coupling through a spacer to lysyl-lysyl-lysyl-lysine afforded the corresponding water soluble lipopeptides which showed antagonistic activity against LPS activation of macrophages *in vitro*.

Glycolipids and lipopeptides containing long chain fatty acids are essential components of the bacterial cell wall and often exhibit powerful immunomodulatory activity:[1] this is for example the case for lipopolysaccharides, cord factors, and certain lipopeptides found in gram$^+$ microorganisms. In the course of our research aimed at discovering new low molecular weight immunomodulators we became interested in recently described mycoloylpeptides. Several members of this series were reported to have adjuvant properties[2] but recently, the water soluble derivative **1**[3] was shown to be inactive in promoting differentiation of HL60 cells.[4] We have shown earlier, that starting from the lipid-A structure, successive modifications lead to new series of compounds with lipid A-like activity (**2**, **3**).[5] The structural similarity between the mycoloylpeptides and our own substances led us to evaluate **1** in our test system.



1 **2** **3**

Surprisingly, whereas **1** did not show significant immunostimulatory activity, in our hands it behaved as a potent antagonist of several lipopolysaccharide (LPS) effects.[6] As a prerequisite to further studies we felt it necessary to examine the role of the stereochemistry of the two chiral centers in the mycolic acid backbone (mixture of racemics in **1**) and decided to synthesize the four possible stereoisomers of 3-O-stearoyl C_{36}-corynomycolic acid **4a-d** and the corresponding water soluble derivatives **5a-d**.



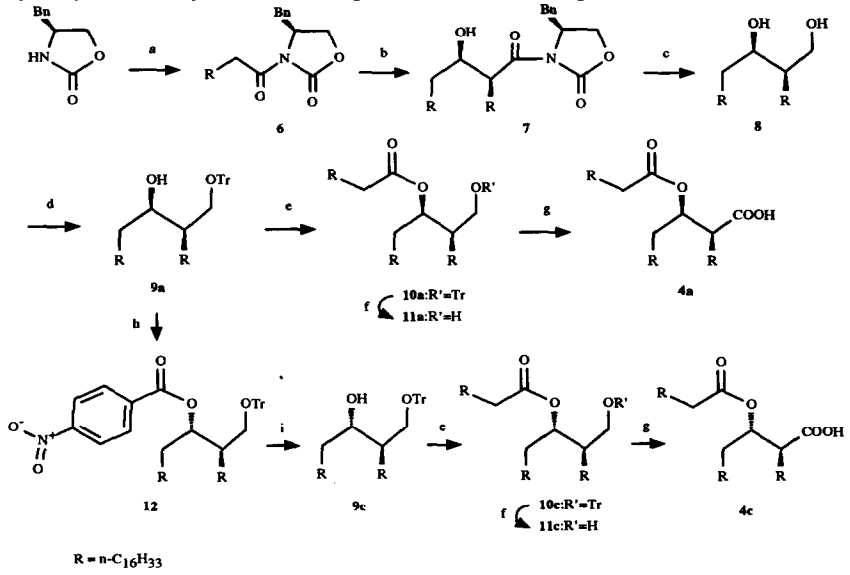
4(a-d) 5(a-d)

To our knowledge, there are only three examples of diastereoselective syntheses of mycolic acids, which rely either on the stereoselective addition of a Grignard reagent on a chiral aldehyde[7] or on the stereoselective alkylation of chiral β-hydroxy esters.[8] The second method provides directly the natural (2*R*, 3*R*)- isomer or its enantiomer but does not allow a ready access to the (2*R*, 3*S*)- and (2*S*, 3*R*)- isomers. The first method gives access to all four isomers, but is somewhat lenghthy. We thought an alternative, in principle very short approach, in which the *syn*-(2,3)- relationship as found in **4a** and **4b** is established in two steps starting from stearic acid, stearaldehyde and a chiral oxazolidinone, according to Evans.[9] Formally, the *anti*-(2,3)-isomers can then be obtained by inversion at C-3. The realization of this approach is shown in scheme **1**. The chiral (*S*)-N-acyl-oxazolidinone **6** was obtained by sequential treatment of (*S*)-4-benzyl-2-oxazolidinone with butyllithium and stearoyl chloride. Treatment of **6** with 9-borabicyclo[3.3.1]nonyl trifluoromethanesulfonate (9-BBN-triflate) and condensation of the resulting boron enolate with 1-octadecanal afforded the adduct **7** in 85% yield, with excellent enantioselectivity.[10] To our surprise, cleavage of **7** proved to be difficult and required carefully adjusted conditions. Our initial attempts to non-reductively cleave the imide function ($LiOH/H_2O$, $LiOH/H_2O_2$) failed to provide the expected β-hydroxy acid and led instead to complex mixtures of unidentified materials. Most of the reductive methods we used were also unsuccessful and in some cases, led to exclusive formation of an unidentified product resulting from opening of the oxazolidinone ring. Finally, we found that **7** could be very cleanly converted to **8** by treatment with $LiBH_4$ in moist ether.[11] Selective protection of the primary hydroxyl group as a trityl ether and acylation of the secondary hydroxyl group in **8** provided **10a**. Cleavage of the trityl group to afford alcohol **11a** was complicated by the facile intramolecular migration of the 3-acyl group to position 1.[12] Fortunately, although the 3- and 1-acyl isomers could not be separated, oxidation of the crude mixture led cleanly to carboxylic acid **4a** easily separated from less polar contaminants.

With the monoprotected diol **9a** in hand, access to the the *anti*-(2*S*,3*S*)- isomer **10c** seemed obvious. In fact, inversion of the stereochemistry at C-3 in **9a** proved to be particularly troublesome and a variety of methods failed to afford even small amounts of the expected compounds.[13] The desired transformation could only be achieved using the recently described conditions for sluggish Mitsunobu reactions.[14] Thus, reaction of **9a** with p-nitro- (or o-nitro-) benzoic acid in the presence of PPh_3 and diethyl azodicarboxylate (DEAD), proceeded smoothly to give **12** in fair yield. Cleavage of the ester linkage required drastic conditions (2M $NaOCH_3$ in methanol [MeOH], 24 h) but cleanly afforded **9c** in high yield which was then converted into **4c** as described for **4a**. The same reaction sequence, starting from (*R*)-4-benzyl-2-oxazolidinone led to **6'**, **7'**, **8'** and **12'**, the enantiomers of **6**, **7**, **8** and **12** respectively, and the **4b** - **11b** *syn*-(2*R*, 3*S*) and **4b** - **11b** *anti*-(2*R*, 3*R*) series.

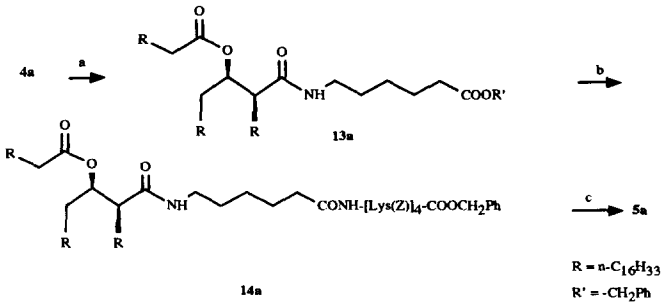
The very low water solubility of **4a-d** prevented their biological testing in cell culture, and it became clear that water soluble derivatives were needed for the planned biological evaluation. Previous experience,[15] led us to consider that the biological activity of **1** in our test system was primarily linked to its lipophilic moiety

and that the contribution of the peptide part of the molecule, if any, was only minor. We decided therefore to prepare compounds **5a-d** in which the assumed active lipophilic part of the molecule is linked to a hydrophilic moiety through a spacer. The synthesis of **5a** depicted in scheme 2 is representative.



(a) (1) BuLi, THF, -78°C, 10 min; (2) $C_{17}H_{35}COCl$, -78°C to r.t., 30 min, 80%; (b) (1) 9-BBN-OTf, N,N-diisopropyl-ethylamine, CH_2Cl_2, 0°C, 30 min; (2) $C_{17}H_{35}CHO$, -78°C, 30 min then r.t., 1.5 h; (3) CH_3OH (excess), 30% H_2O_2 (excess), buffer pH7, 0°C, 1 h, 82%; (c) (1) $LiBH_4$, ether, r.t., 15 min; (2) NH_4Cl, 0°C, 83%; (d) Trityl chloride, pyridine, 90%; (e) $C_{17}H_{35}COCl$, pyridine / CH_2Cl_2, 0°C, 87% (**10a**) or 85% (**10c**); (f) HCOOH / ether 1:1, r.t., 1.5 h, 62% (mixture containing **11a**, 88% and 2-hexadecyl-1-octadecanoyloxyeicosan-3-ol, 12%) or 76% (mixture containing **11c**, 90% and 2-hexadecyl-1-octadecanoyloxyeicosan-3-ol, 10%); (g) Aliquat^R 336, $KMnO_4$, hexane / acetic acid / water, r.t., 20 h, 70% (**4a**) or 60% (**4c**); (h) DEAD, PPh_3, p-$NO_2C_6H_4COOH$, benzene, r.t., 5 h, 63 %; (i) NaOMe (2M / MeOH), r.t., 96%.

Scheme 1



(a) (1) DCC, N-hydroxysuccinimide, CH_2Cl_2, 0°C, 10 min then r.t., 30 min; (2) $Cl^- NH_3^+$-$(CH_2)_5$-$COOCH_2Ph$, N,N-diisopropyl-ethylamine, DMF / CH_2Cl_2, r.t., 24 h, 76%; (b) (1) Pd 10% / C, H_2, ethyl acetate; (2) DCC, N-hydroxysuccinimide, CH_2Cl_2, 0°C, 3 h; (3) $Cl^- NH_3^+$-[Lys(Z)$_4$]-$COOCH_2Ph$, CH_2Cl_2, r.t., 16 h, 32%; (c) Pd 10% / C, H_2, THF / 0.1 N HCl, 88%

Scheme 2

When evaluated for their antagonistic activity against LPS, the four lipopeptides **5a-d** were found to be highly active and equivalent to **1** thus confirming our hypothesis that the peptide moiety in the latter plays a minor role regarding LPS antagonism aspects. Surprisingly, the biological activity of the new compounds in our assays did not seem to be dependent on their stereochemistry. Detailed results of the biological studies with **5a-d** will be published elsewhere.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 250 MHz (or 500 MHz when indicated) using a Bruker AC-250 or Bruker AMX-500 spectrometer. FAB mass spectra were determined on a VG 70-SE spectrometer and optical rotations measurements were performed on a Perkin-Elmer 141 polarimeter. Combustion analysis was performed by the Analytical Department, Sandoz Pharma. For thin layer chromatography we used Silica Gel 60 F_{254} plates (Merck) and column chromatography was performed using E.Merck Kieselgel 60 (230 - 400 mesh).

(S)-3-Octadecanoyl-4-phenyloxazolidin-2-one (**6**). To a cooled (-78°C) solution of (*S*)-4-phenyl-oxazolidin-2-one (3.544 g, 20 mmol), in tetrahydrofuran (THF, 100 mL), was added dropwise under argon (Ar) a solution of BuLi in hexane (1.6 M, 12.5 mL, 20 mmol). The clear solution was stirred for 10 min and octadecanoyl chloride (6.059 g, 20 mmol) dissolved in THF (50 mL) was added. The mixture was allowed to reach room temperature and partitioned between hexane (300 mL) and water (300 mL). The organic phase was dried and concentrated *in vacuo*. Polar contaminants were removed by filtration through a plug of silica gel (eluting with toluene) and the crude material thus obtained was recrystallized from MeOH to afford pure **6** (7.079 g, 80%): mp 63 - 65°C; $[\alpha]_D^{20}$ +33° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 6.3 Hz, 3 H), 1.2 - 1.5 (broad, 28 H), 1.70 (m, 2 H), 2.77 (dd, *J* = 13.4, 9.6 Hz, 1 H), 2.94 (m, 2 H), 3.31 (dd, *J* = 13.4, 3.3 Hz, 1 H), 3.92 - 4.05 (m, 2 H), 4.67 (m, 1 H), 7.15 - 7.40 (m, 5 H); FAB m.s.: m/z 444 (MH^+). *Anal.* Calc. for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22; N, 3.16; Found: C, 75.5; H, 10.3; N, 3.3.

(R)-3-Octadecanoyl-4-phenyloxazolidin-2-one (**6'**). Preparation as for **6**. Yield: 85%: mp 63 - 65°C; $[\alpha]_D^{20}$ -33.5° (*c* 1, $CHCl_3$); FAB m.s.: m/z 444 (MH^+). *Anal.* Calc. for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22; N, 3.16 ; Found: C, 75.9; H, 10.5; N, 2.8.

(S)-3-[(2S,3R)-2-Hexadecyleicosanoyl-3-hydroxy]-4-phenyloxazolidin-2-one (**7**). The acyl oxazolidinone **6** (7.022 g, 15.83 mmol), was dissolved in CH_2Cl_2 (100 mL). The solution was cooled to 0°C and placed under Ar. 9-BBN-OTf (4.702 g, 17.41 mmol) and diisopropylethylamine (2.248 g, 17.41 mmol) were added. After stirring for 30 min the solution was cooled to -78°C and octadecanal (4.674 g, 17.41 mmol) dissolved in CH_2Cl_2 (45 mL) was added. The mixture was stirred 30 min at -78°C and 1.5 h at room temperature (r.t.). MeOH (80 mL) and pH7 buffer (32 mL) were added, the mixture was cooled to 0°C, and H_2O_2 (30%, 40 mL) was added. After 1 h the mixture was poured into water (700 mL) and extracted with CH_2Cl_2 (700 mL). The organic phase was washed once with water, dried and concentrated *in vacuo*. At this point, TLC showed a major spot accompanied by a slightly lower migrating minor one. Chromatography (eluant: toluene / ethyl acetate 92:8) afforded the pure (*2S,3R*)-compound **7** (9.231g, 82%) and a mixture (1.215 g) containing **7** (43%) and its (*2R,3S*)-isomer (57%). The overall *(syn)*-isomers ratio as determined by proton magnetic resonance spectroscopy (^{1}H NMR) was 93:7. The two other possible (*anti*)-isomers, if formed, represented less than 0.5 % of the mixture. $[\alpha]_D^{20}$ +16.2° (*c* 1, $CHCl_3$); ^{1}H NMR (500 Mhz, $CDCl_3$): δ 0.88 (two overlapping t, *J* = 6.5 Hz, 6 H), 1.15 - 1.4 (broad, 58 H), 1.50 (broad, 2 H), 1.61 (m, 1 H), 1.87 (m, 1 H), 2.44 (d, *J* = 3.3 Hz, D_2O exchanged, 1 H), 2.71 (dd, *J* = 13.2, 10.0 Hz, 1 H), 3.36 (dd, *J* = 13.2, 3.3 Hz, 1 H), 3.87 (m, 1 H), 4.07 (dt, *J* = 9.8, 4 Hz, 1H), 4,15 - 4.25 (m, 2 H), 4.74 (m, 1 H), 7.15 - 7.40 (m, 5 H). *Anal.* Calc. for $C_{46}H_{81}NO_4$: C, 77.58; H, 11.46; N, 1.97; Found: C, 77.6; H, 11.8; N, 1.5.

Mixture of **7** *and its (2R,3S)-isomer.* ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (two overlapping t, *J* = 6.5 Hz, 6 H), 1.15 - 1.4 (broad, 58 H), 1.50 - 1.90 (m, 4 H), 2.41 (d, *J* = 3.4 Hz, 0.40 H), 2.47 (d, *J* = 9 Hz, 0.59 H), 2.71 (dd, *J* = 13.2, 10.0 Hz, 1 H), 3.36 (two overlapping dd, 1 H), 3.73 (m, 0.64 H), 3.87 (m, 0.46 H), 3.94 (dt, *J* = 8.1, 6 Hz, 0.63 H), 4.07 (dt, *J* = 9.8, 4 Hz, 0.44 H), 4.15 - 4.25 (m, 2 H), 4.70 - 4.80 (two overlapping m, 1 H), 7.15 - 7.40 (m, 5 H).

(R)-3-[(2R,3S)-2-Hexadecyleicosanoyl-3-hydroxy]-4-phenyloxazolidin-2-one (**7'**). Preparation as for **7**. Ratio *(2R,3S) / (2S,3R)* 95:5. Yield: 78%. $[\alpha]_D^{20}$ -17.2° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{46}H_{81}NO_4$: C, 77.58; H, 11.46; N, 1.97; Found: C, 77.4; H, 11.7; N, 2.

(2R,3S)-2-Hexadecyleicosan-1,3-diol (**8**). The adduct **7** (4.085 g, 5.73 mmol) was dissolved in ether (250 mL, not dried) and $LiBH_4$ (2.505 g) was added. The resulting suspension was stirred at r.t. for 15 min, cooled to 0°C and a saturated solution of NH_4Cl (200 mL) was slowly added. The organic phase was dried, and concentrated under reduced pressure. Chromatography of the residue (eluant: toluene / ethyl acetate 4:1)

yielded pure **8** (2.570 g, 83%) as a colorless wax. $[\alpha]_D^{20}$ -0.5°, $[\alpha]_{436}^{20}$ -3.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (two overlapping t, *J* = 6.3 Hz, 6 H), 1.15 - 1.5 (broad, 62 H), 1.62 (m, 1 H), 2.26 (d, *J* = 4.8 Hz, D_2O exchanged, 1 H), 2.37 (t, *J* = 4.9 Hz, D_2O exchanged, 1 H), 3.65 - 3.85 (m, 3 H). *Anal.* Calc. for $C_{36}H_{74}O_2$: C, 80.22; H, 13.84; Found: C, 80.4; H, 14.1.

(2S,3R)-2-Hexadecyleicosan-1,3-diol (**8'**). Preparation as for **8**. Yield: 87%. $[\alpha]_D^{20}$ +1.3°, $[\alpha]_{436}^{20}$ +2.7° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{36}H_{74}O_2$: C, 80.22; H, 13.84; Found: C, 80.1; H, 14.2.

(2S,3R)-2-Hexadecyl-1-trityloxy-eicosan-3-ol (**9a**). Under Ar, the diol **8** (2.480 g, 4.60 mmol), was dissolved in pyridine (40 mL). Trityl chloride (2.565 g, 9.20 mmol) was added and the yellow solution was stirred at r.t. for 36 h. MeOH (10 mL) was added and stirring was continued for 24 h. The solvents were removed under reduced pressure (remaining traces of pyridine were removed by coevaporating twice with toluene). The residue was taken-up in toluene / hexane 1:1 and the insoluble material was filtered-off. Chromatography (eluant: hexane / toluene 1:1) afforded pure **9a** (3.220 g, 90%). $[\alpha]_D^{20}$ -3°, $[\alpha]_{436}^{20}$ -7.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (two overlapping t, *J* = 6.3 Hz, 6 H), 1.15 - 1.5 (broad, 62 H), 1.68 (m, 1 H), 2.86 (d, *J* = 5.1 Hz, D_2O exchanged, 1 H), 3.16 - 3.30 (m, 2 H), 3.71 (m, 1 H), 7.20 - 7.50 (m, 15 H). *Anal.* Calc. for $C_{55}H_{88}O_2$: C, 84.55; H, 11.35; Found: C, 84.5; H, 11.7.

(2R,3S)-2-Hexadecyl-1-trityloxy-eicosan-3-ol (**9b**). Preparation as for **9a**. Yield: 84%. $[\alpha]_D^{20}$ +3.3°, $[\alpha]_{436}^{20}$ +7.4° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{55}H_{88}O_2$: C, 84.55; H, 11.35; Found: C, 84.9; H, 11.6.

(2R,3S)-2-Hexadecyl-3-octadecanoyloxy-1-trityloxy-eicosane (**10a**). The trityl derivative **9a** (1.200 g, 1.53 mmol), was dissolved in cooled (0°C) pyridine (10 mL). The flask was flushed with Ar and octadecanoyl chloride (0.909 g, 3 mmol) dissolved in CH_2Cl_2 (3 mL) was added. The solution was stirred for 48 h at 0°C. The mixture was poured in CH_2Cl_2 and extracted with 1N HCl. The organic layer was concentrated and the residue was freed from polar contaminants by filtration through a short silica gel column to afford pure **10a** (1.400 g, 87%) as a colorless oil. $[\alpha]_D^{20}$ -1.8°, $[\alpha]_{436}^{20}$ -3.7°(*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, *J* = 6.3 Hz, 9 H), 1.15 - 1.45 (broad, 90 H), 1.55 (m, 2 H), 1.76 (m, 1 H), 2.18 (t, *J* = 7.4 Hz, 2 H), 2.98 - 3.10 (m, 2 H), 5.10 (m, 1 H), 7.18 - 7.50 (m, 15 H). *Anal.* Calc. for $C_{73}H_{122}O_3$: C, 83.68; H, 11.74; Found: C, 84.0; H, 12.0.

(2S,3R)-2-Hexadecyl-3-octadecanoyloxy-1-trityloxy-eicosane (**10b**). Preparation as for **10a**. Yield: 89%. $[\alpha]_D^{20}$ +1.8°, $[\alpha]_{436}^{20}$ +3.5° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{73}H_{122}O_3$: C, 83.68; H, 11.74; Found: C, 83.5; H, 11.9.

(2R,3R)-2-Hexadecyl-3-octadecanoyloxy-1-trityloxy-eicosane (**10c**). Preparation as for **10a**. Yield: 85%. $[\alpha]_D^{20}$ +6.6°, $[\alpha]_{436}^{20}$ +14° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, *J* = 6.3 Hz, 9 H), 1.15 - 1.40 (broad, 88 H), 1.40 - 1.60 (m, 4 H), 1.86 (m, 1 H), 2.18 (t, *J* = 7.4 Hz, 2 H), 3.00 - 3.13 (m, 2 H), 5.10 (m, 1 H), 7.18 - 7.50 (m, 15 H). *Anal.* Calc. for $C_{73}H_{122}O_3$: C, 83.68; H, 11.74; Found: C, 83.6; H, 11.8.

(2S,3S)-2-Hexadecyl-3-octadecanoyloxy-1-trityloxy-eicosane (**10d**). Preparation as for **10a**. Yield: 86%. $[\alpha]_D^{20}$ -7°, $[\alpha]_{436}^{20}$ -13.8° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{73}H_{122}O_3$: C, 83.68; H, 11.74; Found: C, 83.5; H, 11.7.

(2R,3S)-2-Hexadecyl-3-octadecanoyloxyeicosan-1-ol (**11a**). To the compound **10a** (0.500 g, 0.48 mmol), was added a solution of HCOOH in ether (50%, 25 mL) and the mixture was stirred at r.t. for 2.5 h under Ar atmosphere. CH_2Cl_2 (200 mL) was added and the solution was carefully shaken with a saturated solution of $NaHCO_3$ (300 mL). The organic layer was dried and concentrated. TLC analysis of the residue showed two main components with different polarities which were separated by column chromatography (eluant: toluene). The faster migrating component (formyl ester of **11a**) was discarded. The other component (240 mg, 62%) was a mixture containing **11a** (88%), and ca. 12%[16] of 2-hexadecyl-1-octadecanoyloxyeicosan-3-ol and was used directly for the next step. ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, *J* = 6.3 Hz, 9 H), 1.15 - 1.40 (broad, 88 H), 1.40 - 1.75 (m, 5 H), 2.15 - 2.20 (two overlapping t, *J* = 7.4 Hz, 2 H), 2.70 (two overlapping d, *J* = 9.1 and 4.6 Hz, 1 H), 3.25 (m, 0.87 H), 3.55 - 3.70 (m, 1 H), 4.06 (dd, *J* = 11.1, 4.7 Hz, 0.12 H), 4.21 (dd, *J* = 11.3, 11.1 Hz, 0.13 H), 5.12 (m, 0.88 H)

(2S,3R)-2-Hexadecyl-3-octadecanoyloxyeicosan-1-ol (**11b**). Preparation as for **11a**. Yield: 75%. Percentage 1-O-acyl ca. 10%.

(2R,3R)-2-Hexadecyl-3-octadecanoyloxyeicosan-1-ol (**11c**). Preparation as for **11a**. Yield: 84%. Percentage 1-O-acyl ca. 10%.

(2S,3S)-2-Hexadecyl-3-octadecanoyloxyeicosan-1-ol (**11d**). Preparation as for **11a**. Yield: 80%. Percentage 1-O-acyl ca. 5%.

(2S,3R)-2-Hexadecyl-3-octadecanoyloxyeicosanoic acid (**4a**). A mixture of the crude **11a** (0.200 g, 0.25 mmol), Aliquat^R 336 (1.00 g) and $KMnO_4$ (0.900g) in hexane (15 mL) and CH_3COOH (3 mL) was stirred at r.t. for 20 h. The mixture was cooled to 0°C and treated with $NaHSO_3$ (2 g) in water (50 mL), then poured into hexane (200 mL). The organic layer was washed with water (200 mL), dried and concentrated. Chromatography (eluant: toluene / ethyl acetate / CH_3COOH 90:9:1), and Bligh-Dyer extraction[17] of the residue afforded pure **4a**: 0.125 g, (70% calculated from **11a** contained in the starting mixture of isomers). $[\alpha]_D^{20}$ +3.28°, $[\alpha]_{436}^{20}$ +6.8° (*c* 0.5, $CHCl_3$); δ ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, *J* = 6.3 Hz, 9 H),

1.15 - 1.70 (101 H), 2.30 (t, J = 7.4 Hz, 2 H), 2.62 (m, 1 H), 5.09 (m, 1 H); FAB m.s.: m/z 819 (MH^+), 535 (100%), 517. *Anal.* Calc. for $C_{54}H_{106}O_4$: C, 79.15; H, 13.04; Found: C, 78.8; H, 12.4.

(2R,3S)-2-Hexadecyl-3-octadecanoyloxyeicosanoic acid (**4b**). Preparation as for **4a**. Yield: 67% (calculated from **11b** contained in the starting mixture of isomers). $[\alpha]_D^{20}$ - 4°, $[\alpha]_{436}^{20}$ -7° (*c* 0.5, $CHCl_3$). *Anal.* Calc. for $C_{54}H_{106}O_4$: C, 79.15; H, 13.04; Found: C, 79.0; H, 12.5.

(2S,3S)-2-Hexadecyl-3-octadecanoyloxyeicosanoic acid: (-)-C_{36}-corynomycolic acid (**4c**). Preparation as for **4a**. Yield: 63%. $[\alpha]_D^{20}$ - 6.6°, $[\alpha]_{436}^{20}$ -12° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, J = 6.3 Hz, 9 H), 1.15 - 1.70 (101 H), 2.29 (t, J = 7.4 Hz, 2 H), 2.62 (m, 1 H), 5.12 (m, 1 H); FAB m.s.: m/z 819 (MH^+), 535 (100%), 517. *Anal.* Calc. for $C_{54}H_{106}O_4$: C, 79.15; H, 13.04; Found: C, 79.4; H, 13.3.

(2R,3R)-2-Hexadecyl-3-octadecanoyloxyeicosanoic acid: (+)-C_{36}-corynomycolic acid (**4d**). Preparation as for **4a**. Yield: 58%. $[\alpha]_D^{20}$ + 6.4°, $[\alpha]_{436}^{20}$ +13.2° (*c* 0.5, $CHCl_3$). *Anal.* Calc. for $C_{54}H_{106}O_4$: C, 79.15; H, 13.04; Found: C, 79.0; H, 13.2.

(2R,3R)-2-Hexadecyl-3-(4-nitrobenzoyloxy)-1-trityloxy-eicosane (**12**). To a solution of **9a** (1.580 g, 2.02 mmol), 4-nitrobenzoic acid (2.340g, 14 mmol) and PPh_3 (3.672 g, 14 mmol) in dry benzene (40 mL), was added dropwise DEAD (2.438 g, 14 mmol). The mixture was stirred for 20 h and concentrated *in vacuo*. The residue was chromatographed (eluant: hexane / toluene 2:1) to afford **12** (1.180 g, 63%). $[\alpha]_D^{20}$ -0.8°, $[\alpha]_{436}^{20}$ -1.4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (2 overlapping t, J = 6.3 Hz, 6 H), 1.05 - 1.50 (broad, 60 H), 1.66 (m, 2 H), 2.05 (m, 1 H), 3.10 - 3.28 (m, 2 H), 5.39 (m, 1 H), 7.18 - 7.50 (m, 15 H), 8.04 (d, J = 8.6 Hz, 2 H), 8.23 (d, J = 8.6 Hz, 2 H). *Anal.* Calc. for $C_{62}H_{91}NO_5$: C, 80.10; H, 9.79; N, 1.51; Found: C, 79.9; H, 9.9; N, 1.6.

(2S,3S)-2-Hexadecyl-3-(4-nitrobenzoyloxy)-1-trityloxy-eicosane (**12'**). Prepared starting from **9c** as described for **12**. Yield: 63%. $[\alpha]_D^{20}$ 0°, $[\alpha]_{436}^{20}$ +0.8° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{62}H_{91}NO_5$: C, 80.10; H, 9.79; N, 1.51; Found: C, 80.2; H, 10.1; N, 1.2.

(2S,3S)-2-Hexadecyl-1-trityloxy-eicosan-3-ol (**9c**). A suspension of **12** (1.000 g), in MeONa/MeOH (2M), was stirred at room temperature for 10 h. The clear solution thus obtained was partitioned between hexane (200 mL) and water (200 mL). The organic phase was dried and concentrated. Chromatography (eluant: hexane/toluene 3:2) gave **9c** (0.810 g, 96%). $[\alpha]_D^{20}$ -5.6°, $[\alpha]_{436}^{20}$ -9.4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (two overlapping t, J = 6.3 Hz, 6 H), 1.15 - 1.80 (63 H), 2.87 (d, J = 6.1 Hz, D_2O exchanged, 1 H), 3.22 (dd, J = 10, 4.3 Hz), 3.30 (dd, J = 10, 2.5 Hz), 3.56 (m, 1 H), 7.20 - 7.50 (m, 15 H). *Anal.* Calc. for $C_{55}H_{88}O_2$: C, 84.55; H, 11.35; Found: C, 84.9; H, 11.6.

(2R,3R)-2-Hexadecyl-1-trityloxy-eicosan-3-ol (**9d**). Prepared starting from **12** as described for **9c**. Yield: 90%. $[\alpha]_D^{20}$ +5.6°, $[\alpha]_{436}^{20}$ +10.5° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{55}H_{88}O_2$: C, 84.55; H, 11.35; Found: C, 84.05; H, 11.21.

6-[(2S,3R)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoic acid benzyl ester (**13a**). The acid **4a** (0.444 g, 0.54 mmol) was dissolved in cool (0°C) CH_2Cl_2 (25 mL). N-Hydroxysuccinimide (0.069 g, 0.6 mmol) and 1,3-dicyclohexylcarbodiimide (DCC, 0.124 g, 0.6 mmol) were added and the reaction was allowed to proceed for 10 min at 0°C and 30 min at r.t.. The precipitated dicyclohexylurea was removed by filtration, the solvent was removed under reduced pressure and the residue was dissolved in dimethylformamide (DMF, 10 mL) and CH_2Cl_2 (5 mL). Benzyl 6-amino-hexanoate tosylate (0.315 g, 0.8 mmol) was added and the pH was adjusted to ca. 8 with diisopropylethylamine. The mixture was stirred at r.t. for 16 h then partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The organic layer was dried, concentrated *in vacuo* and the residue was chromatographed (eluant: toluene / ethyl acetate 93 : 1) to afford **13a** (0.420 g, 76%).$[\alpha]_D^{20}$ +4.2°, $[\alpha]_{436}^{20}$ +7.5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, J = 6.3 Hz, 9 H), 1.0 - 1.8 (98 H), 2.20 - 2.42 (m, 5 H), 3.24 (m, 2 H), 5.00 (pseudo q, 1 H), 5.12 (s, 2 H), 5.53 (t, J = 5.8 Hz, 1 H), 7.36 (m, 5 H). *Anal.* Calc. for $C_{67}H_{123}NO_5$: C, 78.69; H, 12.12; N, 1.37; Found: C, 78.6; H, 12.0; N, 1.4.

6-[(2R,3S)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoic acid benzyl ester (**13b**). Prepared as **13a**. Yield: 66%. $[\alpha]_D^{20}$ -4.0°, $[\alpha]_{436}^{20}$ -8.6° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{67}H_{123}NO_5$: C, 78.69; H, 12.12; N, 1.37; Found: C, 78.4; H, 12.0; N, 1.5.

6-[(2S,3S)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoic acid benzyl ester (**13c**). Prepared as **13a**. Yield: 68%. $[\alpha]_D^{20}$ -3.7°, $[\alpha]_{436}^{20}$ -6.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (3 overlapping t, J = 6.3 Hz, 9 H), 1.0 - 1.8 (98 H), 2.20 - 2.42 (m, 5 H), 3.21 (m, 2 H), 4.98 (m, 1 H), 5.12 (s, 2 H), 5.74 (t, J = 5.7 Hz), 7.36, (m, 5 H). *Anal.* Calc. for $C_{67}H_{123}NO_5$: C, 78.69; H, 12.12; N, 1.37; Found: C, 78.1; H, 11.8; N, 1.4.

6-[(2R,3R)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoic acid benzyl ester (**13d**). Prepared as **13a**. Yield: 67%. $[\alpha]_D^{20}$ +2.9°, $[\alpha]_{436}^{20}$ +4.6° (*c* 1, $CHCl_3$). *Anal.* Calc. for $C_{67}H_{123}NO_5$: C, 78.69; H, 12.12; N, 1.37; Found: C, 78.6; H, 12.0; N, 1.4.

N^2-[N^2-[N^2-[N^2-[6-[(2S,3R)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-N^6-(benzyloxycarbonyl)-L-lysyl]-N^6-(benzyloxycarbonyl)-L-lysyl]-N^6-(benzyloxycarbonyl)-L-lysyl]-N^6-(benzyloxycarbonyl)-L-lysine benzyl ester (**14a**). **13a** (0.370 g, 0.362 mmol) was dissolved in ethyl acetate (300 mL) and hydrogenated over 10% Pd / C (0.050 g) for 1 h. The catalyst was removed by filtration, well washed with a mixture of $CHCl_3$ / MeOH 9:1, and the solvent was evaporated under reduced pressure. The residue was

dissolved in cooled (0°C) CH_2Cl_2 (60 mL), N-hydroxysuccinimide (0.046 g, 0.4 mmol) and DCC (0.083 g, 0.4 mmol) were added and the mixture was stirred for 3 h. NH_2-[Lys-(Z)]$_4$-OCH_2Ph[18] (0.463 g, 0.4 mmol) was added, the pH was adjusted to ca. 7.5 with triethylamine and stirring was continued for 16 h at room temperature. The solvent was evaporated and the residue was purified by chromatography (eluant: toluene / ethyl acetate 1:1 then $CHCl_3$ / MeOH 95:5) and Bligh-Dyer extraction: 0.243 g (32%). $[\alpha]_D^{20}$ -12.8° (*c* 1, $CHCl_3$ / MeOH 1:1); ^{1}H NMR (500 MHz, $CDCl_3$ / CD_3OD 9:1) δ 0.88 (3 overlapping t, *J* = 6.8 Hz, 9 H), 1.15 - 1.9 (122 H), 2.17 (m, 2 H), 2.30 (t, *J* = 6.8 Hz, 2 H overlapping with m, 1 H), 3.04 - 3.24 (m, 10 H), 4.10 - 4.70 (broad, 4 H), 5.00 (pseudo q, 1 H), 5.04 - 5.20 (m, 10 H), 7.23 - 7.38, (m, 25 H). *Anal.* Calc. for $C_{123}H_{195}N_9O_{17}$: C, 71.30; H, 9.49; N, 6.08; Found: C, 71.4; H, 9.2; N, 6.0.

N²-[N²-[N²-[N²-[6-[(2R,3S)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-N⁶-(benzyloxycarbonyl)-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysine benzyl ester* (**14b**). Prepared as **14a**. Yield: 54%. $[\alpha]_D^{20}$ -18.3° (*c* 0.3, $CHCl_3$ / MeOH 1:1); ^{1}H NMR (500 MHz, $CDCl_3$ / CD_3OD 9:1) δ 0.88 (3 overlapping t, *J* = 6.8 Hz, 9 H), 1.15 - 1.9 (122 H), 2.17 (m, 2 H), 2.30 (q, *J* = 7.6, 6.7 Hz, 2 H, overlapping with m, 1 H), 3.04 - 3.24 (m, 10 H), 4.10 - 4.70 (broad, 4 H), 5.01 (pseudo q, 1 H), 5.04 - 5.20 (m, 10 H), 7.23 - 7.38, (m, 25 H). *Anal.* Calc. for $C_{123}H_{195}N_9O_{17}$: C, 71.30; H, 9.49; N, 6.08; Found: C, 71.5; H, 9.2; N, 6.1.

N²-[N²-[N²-[N²-[6-[(2S,3S)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-N⁶-(benzyloxycarbonyl)-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysine benzyl ester* (**14c**). Prepared as **14a**. Yield: 63%. $[\alpha]_D^{20}$ -19.8° (*c* 1, $CHCl_3$ / MeOH 1:1); ^{1}H NMR (500 MHz, $CDCl_3$ / CD_3OD 9:1) δ 0.88 (3 overlapping t, *J* = 6.8 Hz, 9 H), 1.15 - 1.9 (122 H), 2.17 (m, 2 H), 2.29 (t, *J* = 7.4 Hz, 2 H), 2.38 (m, 1 H), 3.05 - 3.25 (m, 10 H), 4.10 - 4.70 (broad, 4 H), 4.96 (m, 1 H), 5.04 - 5.20 (m, 10 H), 7.23 - 7.38, (m, 25 H). *Anal.* Calc. for $C_{123}H_{195}N_9O_{17}$: C, 71.30; H, 9.49; N, 6.08; Found: C, 71.1; H, 9.6; N, 6.1.

N²-[N²-[N²-[N²-[6-[(2R,3R)-2-Hexadecyl-3-octadecanoyloxyeicosanoylamino]-hexanoyl]-N⁶-(benzyloxycarbonyl)-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysyl]-N⁶-(benzyloxycarbonyl)*-L-*lysine benzyl ester* (**14d**). Prepared as **14a**. Yield: 52%. $[\alpha]_D^{20}$ -10.9° (*c* 1, $CHCl_3$ / MeOH 1:1); ^{1}H NMR (500 MHz, $CDCl_3$ / CD_3OD 9:1) δ 0.88 (3 overlapping t, *J* = 6.8 Hz, 9 H), 1.15 - 1.9 (122 H), 2.17 (m, 2 H), 2.28 (t, *J* = 7.4 Hz, 2 H), 2.37 (m, 1 H), 3.05 - 3.25 (m, 10 H), 4.10 - 4.70 (broad, 4 H), 4.96 (m, 1 H), 5.04 - 5.20 (m, 10 H), 7.23 - 7.38, (m, 25 H). *Anal.* Calc. for $C_{123}H_{195}N_9O_{17}$: C, 71.30; H, 9.49; N, 6.08; Found: C, 71.2; H, 9.5; N, 6.0.

N⁶-[(2S,3R)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-L-*lysyl*-L-*lysyl*-L-*lysyl*-L-*lysine tetrahydrochloride* (**5a**). The protected lipopeptide **14a** (0.203 g, 0.1 mmol) partially dissolved in a THF / 0.1 N aqueous HCl 5:1 (30 mL) was hydrogenated over 10%Pd / C (20 mg) for 8h (eluant for reaction control $CHCl_3$ / CH_3OH / H_2O / CH_3COOH, 125:75:20:10). Most of the catalyst was filtered-off and the last traces of catalyst were removed by filtration through a 0.02 µm filter.[19] The filtrate was lyophilized to afford 137 mg (88%) of **5a**. $[\alpha]_D^{20}$ -8° (*c* 0.5, MeOH / H_2O 1:1); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3 overlapping t, *J* = 6.8 Hz, 9 H), 1.15 - 2.0 (122 H), 2.28 (t, 7.5 Hz, 2 H), 2.38 (t, *J* = 7.1 Hz, 2 H), 2.40 (m, 1 H), 2.9 - 3.0 (m, 8 H), 3.10 - 3.20 (m, 2 H), 4.29 (dd, *J* = 8.8, 5.8 Hz, 1 H), 4.32 - 4.4 (m, 3 H), 5.06 (m, 1 H); FAB m.s.: m/z 1444 (MH^+).

N⁶-[(2R,3S)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-L-*lysyl*-L-*lysyl*-L-*lysyl*-L-*lysine tetrahydrochloride* (**5b**). Prepared as **5a**. Yield: 91%. $[\alpha]_D^{20}$ -38° (*c* 0.5, MeOH / H_2O 1:1); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3 overlapping t, *J* = 6.9 Hz, 9 H), 1.15 - 2.0 (122 H), 2.28 (t, 7.5 Hz, 2 H), 2.33 (t, *J* = 7.1 Hz, 2 H), 2.39 (m, 1 H), 2.9 - 3.0 (m, 8 H), 3.10 - 3.20 (m, 2 H), 4.29 (dd, *J* = 8.8, 5.8 Hz, 1 H), 4.32 - 4.4 (m, 3 H), 5.06 (m, 1 H); FAB m.s.: m/z 1444 (MH^+).

N⁶-[(2S,3S)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-L-*lysyl*-L-*lysyl*-L-*lysyl*-L-*lysine tetrahydrochloride* (**5c**). Prepared as **5a**. Yield 94%. $[\alpha]_D^{20}$ -15° (*c* 0.5, MeOH / H_2O 1:1); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3 overlapping t, *J* = 6.9 Hz, 9 H), 1.15 - 2.0 (122 H), 2.20 - 2.32 (m, 4 H), 2.45 (m, 1 H), 2.9 - 3.0 (m, 8 H), 3.06 - 3.20 (m, 2 H), 4.29 (dd, *J* = 8.6, 5.7 Hz, 1 H), 4.32 - 4.4 (m, 3 H), 5.05 (m, 1 H); FAB m.s.: m/z 1444 (MH^+).

N⁶-[(2R,3R)-2-Hexadecyl-3-octadecanoyloxy-eicosanoylamino]-hexanoyl]-L-*lysyl*-L-*lysyl*-L-*lysyl*-L-*lysine tetrahydrochloride* (**5d**). Prepared as **5a**. Yield 84%. $[\alpha]_D^{20}$ -13° (*c* 0.5, MeOH / H_2O 1:1); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3 overlapping t, *J* = 6.9 Hz, 9 H), 1.15 - 2.0 (122 H), 2.20 - 2.32 (m, 4 H), 2.45 (m, 1 H), 2.9 - 3.0 (m, 8 H), 3.08 - 3.20 (m, 2 H), 4.29 (dd, *J* = 8.8, 5.7 Hz, 1 H), 4.32 - 4.4 (m, 3 H), 5.05 (m, 1 H); FAB m.s.: m/z 1444 (MH^+).

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10. Enantioselectivity was determined by ^{1}H NMR (500 MHz) by comparing the intensities of the signals at δ 4.07 (*CH*-C=O, *S,S,R* or *R,R,S* derivatives) and δ 3.94 (*CH*-C=O, *S,R,S* or *R,S,R* derivatives) in the purified mixture of isomers.
11. The followings attempts were made: Reduction with $LiAlH_4$ in ether or THF (decomposition), $NaBH_4$ in MeOH or moist THF (**8a** was formed along with side products), $LiBH_4$ in dry THF or ether (exclusive formation of an unidentified product resulting from oxazoline ring opening), $LiBH_4$ in ether (Fluka, containing <0.1% H_2O) (clean reduction to **8a**).
12. We were unable to completely avoid the migration of the 3-octadecanoyl group using a variety of conditions. For example attempts to remove the trityl group by hydrogenolysis (Pd 10% / C, hexane) led to mixtures of primary and secondary esters in variable proportions (up to 1:2) as determined by ^{1}H NMR spectroscopy. To definitely prove that the migration had occurred, the mixture was quantitatively converted back to **8a** by treatment with MeONa/MeOH. The trityl group was best removed using formic acid in ether. Even then, ca. 5 - 10% of the 1-O-acyl isomer was formed along with some 1-formate.
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16. The proportion 3-O-acyl / 1-O-acyl was determined by ^{1}H NMR by comparing the intensities of the signals at δ 3.25 (-CH_aH_bOH, 3-O-acyl derivative) and 4.06 (-CH_aH_bOAcyl, 1-O-acyl derivative).
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19. We used Merck's ANOTOP^R 25 0.02 µM disposable syringe filters.

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